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Oxidation of Plasma Cysteine/Cystine and GSH/GSSG Redox Potentials by Acetaminophen and Sulfur Amino Acid Insufficiency in Humans

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ABSTRACT

Variations in plasma sulfur amino acid (SAA) pools are associated with disease risks, but little information is available about the factors affecting plasma SAA pools. Drug metabolism by glutathione (GSH) and sulfate conjugation can, in principle, represent a quantitatively important burden on SAA supply. The present study was designed to determine whether therapeutic doses of acetaminophen (APAP) alter SAA metabolism in healthy human adults. A double-blind, crossover design incorporating four treatment periods with diets providing 100% of the recommended dietary allowance (RDA) for SAA without or with APAP (15 mg/kg) and 0% RDA for SAA without or with APAP, in randomized order. After a 3-day equilibration period, chemically defined diets with 100 or 0% RDA for SAA were given for 2 complete days. On day 3, APAP or placebo was given in two successive doses (6-h interval), and timed plasma samples were collected. With SAA intake at 100% RDA, APAP administration oxidized the plasma cysteine/cystine redox potential (E_hCySS) but not the plasma GSH/GSSG redox potential (E_hGSSG). The extent of oxidation caused by APAP was similar to that seen with 0% SAA and no APAP. However, APAP administration with 0% SAA did not cause further oxidation beyond APAP or 0% SAA alone. In contrast, an oxidation of the plasma E_hGSSG was apparent for SAA insufficiency only with APAP. The results suggest a need to evaluate possible effects of APAP in association with SAA insufficiency as a contributing factor in disease risk.

The sulfur amino acids (SAAs), methionine (Met) and cysteine (Cys), are required to maintain protein synthesis (Young, 2001; Bottiglieri, 2002) and are linked to drug metabolism through use of Cys for biosynthesis of glutathione (GSH) and sulfate (Jones et al., 1995; McCarver and Hines, 2002). Met is nutritionally essential and is a precursor for Cys (Stipanuk and Watford, 2000) so that both are important to Cys supply. Because Cys is irreversibly lost through use of GSH and sulfate for drug metabolism, the requirement for SAA intake during drug metabolism is greater than that normally needed for protein synthesis and turnover (Reicks et al., 1988).

Previous studies of SAA availability and drug metabolism in rodents show that prolonged dietary deficiency of Met and ingestion of APAP significantly decreased body weight and hepatic GSH and Cys concentrations (Reicks et al., 1988; Price and Jollow, 1989). In vitro studies of SAA insufficiency alone showed that severe Cys deficiency inhibited proliferation, decreased GSH concentration, and caused a more oxidized intracellular GSH/GSSG redox potential (E_hGSSG) in human colonic epithelial cells (Miller et al., 2002). Dietary deficiency of SAA also caused a marked decrease in plasma Cys and CySS concentrations and a significant oxidation of E_hCySS values in rats, and this was associated with an increase in plasma GSSG concentration and more oxidized E_hGSSG (Nkabyo et al., 2006). Together, these data suggest that an interaction between limited dietary SAA supply and drug metabolism could occur and be reflected in changes in the plasma Cys and GSH pools.

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ABBREVIATIONS: APAP, acetaminophen; AUC, area under the curve; CySS, cystine; E_h, redox potential; RDA, recommended dietary allowance; SAA, sulfur amino acid; GCRC, General Clinical Research Center; BMI, body mass index; HPLC, high-performance liquid chromatography.

Such effects on plasma Cys and GSH could be important because decreased and/or oxidized plasma GSH and Cys pools have been associated with aging, chronic illness, and disease risk factors, including smoking and alcohol abuse (Moriarty-Craige and Jones, 2004). A diurnal variation study in humans showed that plasma Cys and GSH concentrations and redox potentials vary in association with food intake (Blanco et al., 2007). Comparison of SAA intake and GSH and sulfate conjugation suggest that drug metabolism could affect these variations. For instance, the recommended dietary allowance (RDA) for SAA for an adult male is approximately 1.9 g/day (Food and Nutrition Board, 2005). Approximately 25% of APAP is metabolized through sulfation and GSH conjugation. With a maximal therapeutic dose of 1 g of APAP, a molar equivalent of approximately 0.2 g of Cys would be used for APAP metabolism; four doses per day would consume 0.8 g, or more than half of the RDA. The mean SAA intake in Americans is considerably higher than the RDA so that the effects of use for drug metabolism in the general population are expected to be relatively small. However, SAA intake ranges from <0.3 to >5 g per day (Flagg et al., 1994), and APAP metabolism occurs principally in the liver, which also supplies GSH to the plasma to maintain Cys supply. Consequently, one can anticipate that use of APAP could alter plasma Cys and GSH pools.

The present study was designed to determine whether APAP alters plasma Cys or GSH pools in healthy humans consuming adequate or inadequate dietary SAA intakes. APAP was selected because it is a generally safe and widely used nonprescription medication (Nimni et al., 2007) with metabolism dependent on SAA. The experiment was designed with four study periods for each individual, two with adequate SAA intake in which APAP was compared with placebo and two with a SAA-free diet in which APAP was compared with placebo. Young healthy individuals were studied under conditions where there are no known risks for either APAP use or short-term SAA insufficiency. The specific hypotheses were that therapeutic doses of APAP would result in decreased plasma Cys and GSH and more oxidized E_hCySS and E_hGSSG under conditions of adequate and insufficient SAA intake. As a control, the effect of 2 days of SAA-insufficient diet on plasma pools was also examined. SAA-insufficient diet and APAP treatment each caused decreases in plasma Cys and an oxidation of E_hCySS. Unexpectedly, APAP did not cause a decrease in Cys or an oxidation of E_hCySS under SAA-insufficient conditions.

Materials and Methods

Materials. Bathophenanthroline disulfonate sodium salt, sodium heparin, sodium iodoacetate, dansyl chloride, L-serine, GSH, GSSG, Cys, CySS, and sodium acetate trihydrate were obtained from Sigma-Aldrich (St. Louis, MO). γ-Glutamylglutamate (γ-Glu-Glu) was obtained from MP Biomedicals (Solon, OH). Boric acid, sodium

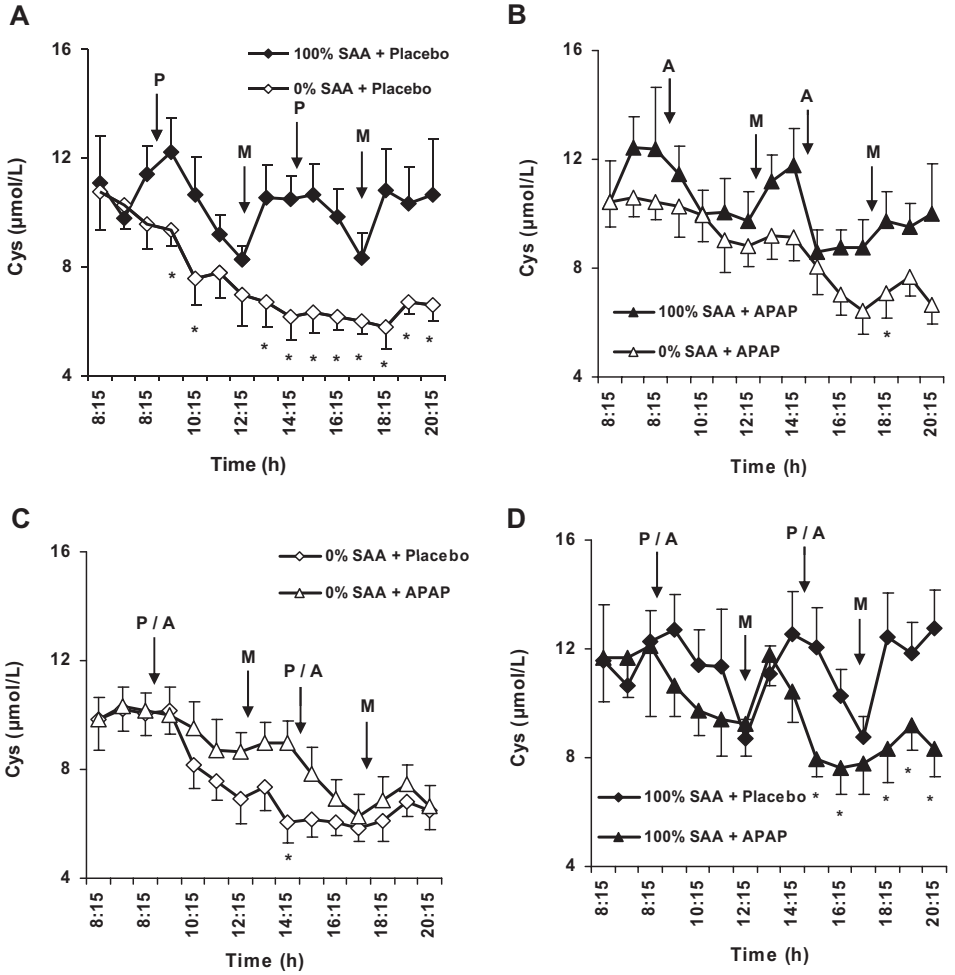

Fig. 1. Effect of SAA insufficiency and APAP administration on plasma Cys concentration in healthy persons aged 18 to 40 years. The experiment was conducted with a 2 × 2 design to evaluate independent effects of SAA and APAP. The times of administration of placebo (P), APAP (A), and meals (M) are indicated by arrows. Data are expressed as means ± S.E. for all subjects at each time point. For paired statistical analyses, data were used only for appropriate pairs as follows: A, 100% SAA + placebo versus 0% SAA + placebo (n = 8); B, 100% SAA + APAP versus 0% SAA + APAP (n = 8); C, 0% SAA + placebo versus 0% SAA + APAP (n = 9); and D, 100% SAA + placebo versus 100% SAA + APAP (n = 10). *, significance at $p < 0.05$.

TABLE 1
Experimental design
Plasma samples were collected hourly for 12 h following administration of APAP (15 mg/kg) or placebo at 8:15 AM on day 6. Study periods described above were randomized for order and conducted at least 1 week apart.

Study Period	Days 1 to 3, Outpatient Equilibration	Days 4 and 5, Inpatient ± SAA	Day 6, Inpatient ± APAP
1	3 Days with 100% RDA for SAA	2 Days in GCRC with 100% RDA for SAA	No APAP
2	3 Days with 100% RDA for SAA	2 Days in GCRC with 100% RDA for SAA	Two sequential doses of APAP
3	3 Days with 100% RDA for SAA	2 Days in GCRC with SAA-free diet	No APAP
4	3 Days with 100% RDA for SAA	2 Days in GCRC with SAA-free diet	Two sequential doses of APAP

tetraborate, potassium tetraborate, perchloric acid, and acetic acid were reagent grade and purchased locally. Methanol, acetone, and chloroform were high-performance liquid chromatography (HPLC) grade.

Human Subjects. This study was reviewed and approved by the Emory Investigational Review Board (501-2004). A total of 12 volunteers, self-described as healthy, were recruited by posting flyers in public locations in the Atlanta/Emory University community. After written informed consent, volunteers were admitted to the outpatient unit of the Emory University Hospital General Clinical Research Center (GCRC) and screened by a physician (T.R.Z.) via medical history, physical examination, fasting standard blood chemistry, urinalysis, and hematology tests. Serum pregnancy tests were performed in females to exclude pregnant individuals. Resting energy expenditure was determined by using indirect calorimetry. Eligibility was established by a body mass index (BMI) < 27, the absence of acute and/or chronic illness, no current smoking history, and age (18–40). Subjects taking nutritional supplements (with the exception of once-daily multivitamin-mineral supplements), antioxidants, or acetaminophen were asked to discontinue use 2 weeks before beginning each study period. With the exception that all females in the study were taking birth control pills, subjects were not taking prescription medications.

Experimental Design. The experimental model was a 2×2 factorial design in which subjects were studied under four different conditions with APAP (0 or 15 mg/kg) and SAA intake (0 or 100% of RDA) (Table 1). Before each inpatient study period, subjects were provided balanced meals of conventional food items containing the RDA for SAA prepared by the GCRC Bionutrition Unit for 3 days (breakfast, lunch, dinner, and snack). Subjects were admitted into the GCRC at 7:00 PM on day 3, and a baseline blood sample was drawn at 8:15 AM on day 4. On days 4 and 5, subjects were given a chemically defined diet containing either 100% SAA or 0% SAA at 8:30 AM, 12:30 PM, 5:30 PM, and 9:30 PM (snack), with a respective distribution of total calories as 30, 30, 30, and 10%. All meals and snacks were consumed over no longer than a 20-min period. On day 6 of each study period, plasma samples were collected hourly for 12 h after administration of APAP (15 mg/kg) or placebo at 8:30 AM and 2:30 PM. On day 6, no morning meal was given to avoid interference with the absorptive phase of acetaminophen. Subsequently, meals were given at 12:30 PM and 5:30 PM. Study subjects served as their own controls to decrease intraindividual variation. The four study periods described in Table 1 were randomized for order and conducted at least 1 week apart.

Diet and Nutrient Intake. The protein equivalents of all diets were supplied in the form of specific L-amino acid mixtures (Ajinomoto USA, Teaneck, NJ), providing 1.0 g/kg per day as outlined in detail (Lyons et al., 2000; Raguso et al., 2000). The standard mixture was patterned after hen's egg protein and provided all nine indispensable (essential) amino acids, including Met, in amounts sufficient for the mean requirements of healthy young adults (Lyons et al., 2000; Raguso et al., 2000), but which are higher than the requirements proposed by the World Health Organization (Di Buono et al., 2001). The standard amino acid mixture also contained eight dispensable (nonessential) amino acids, including Cys and glutamate, and was glutamine- and taurine-free. To compensate for the difference in Met + Cys between the 0 and 100% SAA diets, the amount of all nonessential amino acids was proportionally changed to maintain a constant dietary nitrogen content. The dietary energy was derived mainly from lipid and carbohydrate sources provided in the form of protein-free wheat starch and butter/safflower oil cookies and a sherbet-based drink as outlined previously (Lyons et al., 2000; Raguso et al., 2000). The Cys was added to the 100% SAA sherbet-like drink immediately before consumption to minimize Cys oxidation to CySS. All subjects were highly compliant with research meals as verified by the GCRC Bionutrition Unit staff.

Adequate hydration and vitamin, mineral, and electrolyte requirements were provided to meet or exceed recommended allowances (Raguso et al., 2000). Water intake was ad libitum. All supplements were administered on a regular schedule by the GCRC research nurses. Body weight was determined daily, and vital signs were obtained every 8 h. Low-level activity was allowed and restricted to walking around the GCRC.

TABLE 2
Baseline characteristics of the total study population ($n = 12$)

	Males	Females	p Value
No. of subjects (%)	6 (50)	6 (50)	
Age	25 ± 5.2	25 ± 4.4	0.95
% White	33	33	
BMI	24.6 ± 0.78	23.8 ± 1.8	0.28

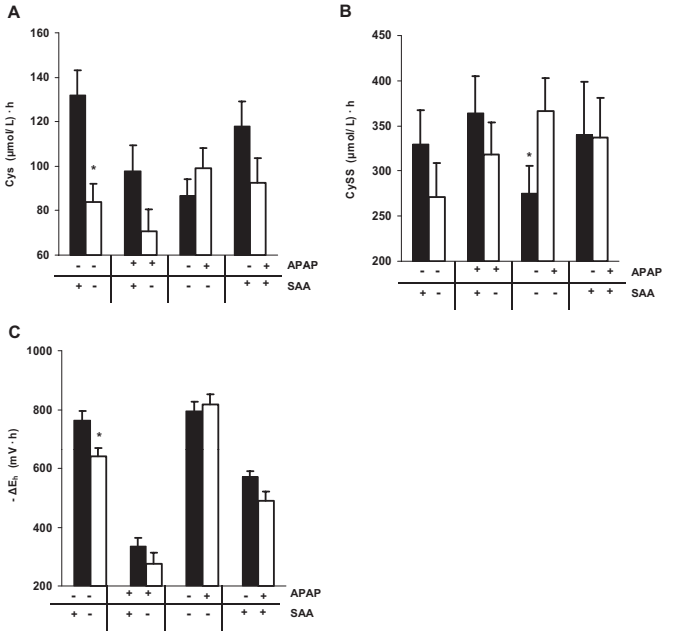

Fig. 2. AUC for plasma Cys and CySS concentrations and Cys/CySS redox potential (E_h CySS) in a 2×2 design to study effects of SAA insufficiency and therapeutic doses of APAP. AUC values were calculated by using the trapezoidal rule for the individual time courses used to create the summary data for: A, Cys (Fig. 1); B, CySS (Fig. 3), and C, E_hCySS (Fig. 4). Data are expressed as means ± S.E. for paired statistical analyses as follows: 100% SAA + placebo versus 0% SAA + placebo, $n = 8$; 100% SAA + APAP versus 0% SAA + APAP, $n = 8$; 0% SAA + placebo versus 0% SAA + APAP, $n = 9$; 100% SAA + placebo versus 100% SAA + APAP, $n = 10$. *, significance at $p < 0.05$.

Sampling and Redox Analysis. Baseline blood samples were drawn at 8:15 AM on days 4 to 6 of each study period. In addition to the baseline blood draw at 8:15 AM, samples were collected hourly through 8:15 PM on day 6 of each study period (Table 1). On day 6, a heparin-lock catheter was placed in a forearm vein for blood sampling. Blood (1.5 ml/liter) was collected and immediately transferred to a microcentrifuge tube containing 0.15 ml of a preservative solution consisting of 0.5 mol of L-serine/liter, 9.3 mmol of bathophenanthroline disulfonate sodium salt/liter, 0.165 mol of γ-glutamylglutamate, 0.4 mol of boric acid, 0.1 mol of sodium borate, 0.144 mol of sodium iodoacetate, and 2.5 mg of sodium heparin/ml (Jones et al., 1998). After centrifugation to pellet cells, supernatant (200 μl) was transferred to microcentrifuge tubes containing 10% ice-cold perchloric acid and 0.2 M boric acid solution. All samples were stored at −80°C until derivatization with dansyl chloride (Jones et al., 1998). HPLC with fluorescence detection was used to quantify dansyl derivatives of Cys, CySS, GSH, and GSSG. These concentrations were used with the Nernst equation to calculate E_h of each redox couple as described (Clarke, 1960; Kirlin et al., 1999; Jones, 2002).

Statistics. Power analysis based on previous studies (Jonas et al., 2000; Lyons et al., 2000; Raguso et al., 2000; Di Buono et al., 2001; Jones et al., 2002) indicated that nine subjects would provide >96% power to detect a relevant SAA-free diet or APAP-induced 4-mV difference in E_hGSSG and >80% to detect a difference of 4 mV in E_hCySS. The study was designed to determine effects of SAA and APAP independently. Not all subjects completed all study periods, so paired *t* tests with Bonferroni correction were used for appropriate comparisons with the number of subjects for each comparison given in the figure legends. We used SPSS software (version 17; SPSS Inc., Chicago, IL) for all analyses. Area under the curve (AUC) values were calculated by using the trapezoidal rule. Results were considered significant at $p \leq 0.05$.

Results

Subject Characteristics. Demographic characteristics of the 12 subjects are summarized in Table 2. The study population was 50% male, and the mean age (± S.D.) was 25 ± 4 years for women and 25 ± 5 years for men. Half of the subjects ($n = 6$) were African American, four were white, one was Hispanic, and one was Native American. All subjects were healthy, having no acute or chronic illness, and none were taking regular prescription medication related to illness. There was no significant difference in ethnicity, age, or BMI between male and female subjects.

Effect of SAA Insufficiency and APAP Administration on Plasma Cys and CySS Concentrations. The details of effects of SAA insufficiency and APAP administration are shown for Cys in Fig. 1, with corresponding AUC data in Fig. 2. The overall conclusion from these complex data are that SAA insufficiency alone results in decreased plasma Cys but that APAP has no effect alone and does not exacerbate the effect of SAA insufficiency. Although significant effects were observed for a few time points with APAP, AUC data (Fig. 2A) showed that APAP administration had no signifi-

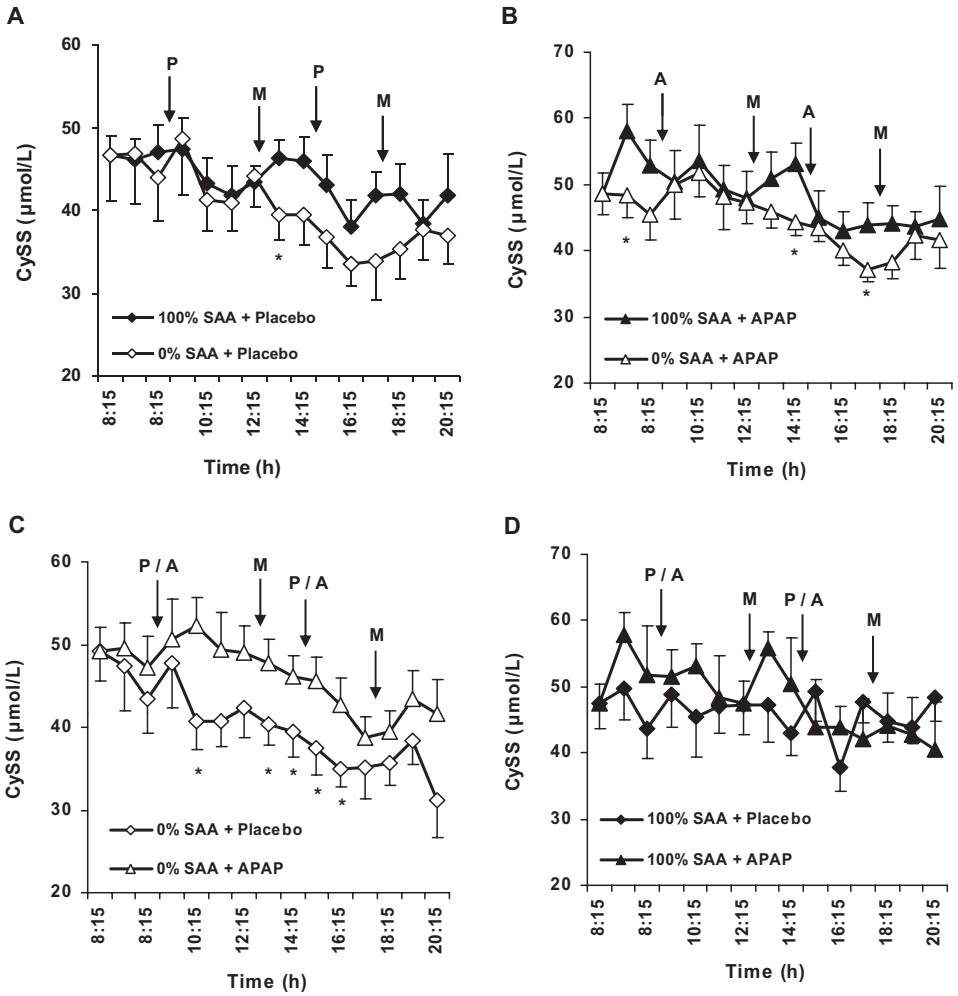

Fig. 3. Effect of SAA insufficiency and APAP administration on plasma CySS concentration in healthy persons aged 18 to 40 years. See legend to Fig. 1 for details. *, significance at $p < 0.05$.

cant effect on plasma Cys concentration in the SAA-replete state (Fig. 1D).

The similarly extensive dataset for CySS is shown in Fig. 3. Despite significant effects at a few time points, AUC data (Fig. 2B) show that an SAA-insufficient diet has, at most, a small effect on plasma CySS. However, a significant increase in plasma CySS concentration was observed with APAP in the SAA-insufficient state (Figs. 2B and 3C). APAP administration did not change plasma CySS concentration in the SAA-repleted state (Figs. 2B and 3D).

Together, the data show that there are effects of SAA insufficiency and APAP on plasma Cys and CySS concentrations, but these effects are not common to all conditions and are not extensive. Most obvious from the complex data, SAA insufficiency decreases Cys without further effect because of APAP, and APAP increases CySS under the condition of SAA insufficiency.

Effect of SAA Insufficiency and APAP Administration on Plasma E_hCySS. Although Cys and CySS concentrations in plasma appear to be determined by transport systems, cell culture studies indicate that mechanisms also exist to regulate the balance of the extracellular concentrations to maintain E_hCySS at approximately −80 mV (Jonas et al., 2002, 2003; Go and Jones, 2005). Calculated values for plasma E_hCySS are shown in Fig. 4 with corresponding AUC data in Fig. 2. Two important observations are apparent from these data: 1) SAA insufficiency alone caused a more oxidized E_hCySS compared with the SAA-adequate diet (Figs. 2C and 4A); and 2) APAP administration alone caused a more oxidized plasma E_hCySS value compared with placebo (Figs. 2C and 4D). Unexpectedly, APAP administration did not cause oxidation of the plasma Cys/CySS pool under SAA-insufficient conditions. Note that the experimental design was to address the effects of SAA and APAP independently and was not powered to address interaction effects.

Effect of SAA Insufficiency and APAP Administration on Plasma GSH and GSSG Concentration. The effects of dietary SAA and APAP on GSH concentration are shown in Fig. 5, with corresponding AUC data in Fig. 6. The two important features that emerge from these data are that plasma GSH was lower with the SAA-insufficient diet only in the presence of APAP, and APAP resulted in lower plasma GSH only with the SAA-insufficient diet. Measurements of GSSG (Fig. 7) showed a few time points were significantly different; however, there were no overall effects on GSSG concentrations apparent from the AUC data (Fig. 6).

Effect of SAA Insufficiency and APAP Administration on Plasma E_hGSSG. The data for E_hGSSG are shown in Fig. 8 with corresponding AUC data in Fig. 6. A few individual comparisons were significantly different (Fig. 8), but the only overall effect apparent from the AUC data was an oxidation of E_hGSSG by the SAA-insufficient diet in the presence of APAP (Fig. 6C). Thus, in contrast to the data for E_hCySS where no interaction between SAA insufficiency and APAP was apparent, the data for E_hGSSG show effects only for SAA insufficiency in the presence of APAP.

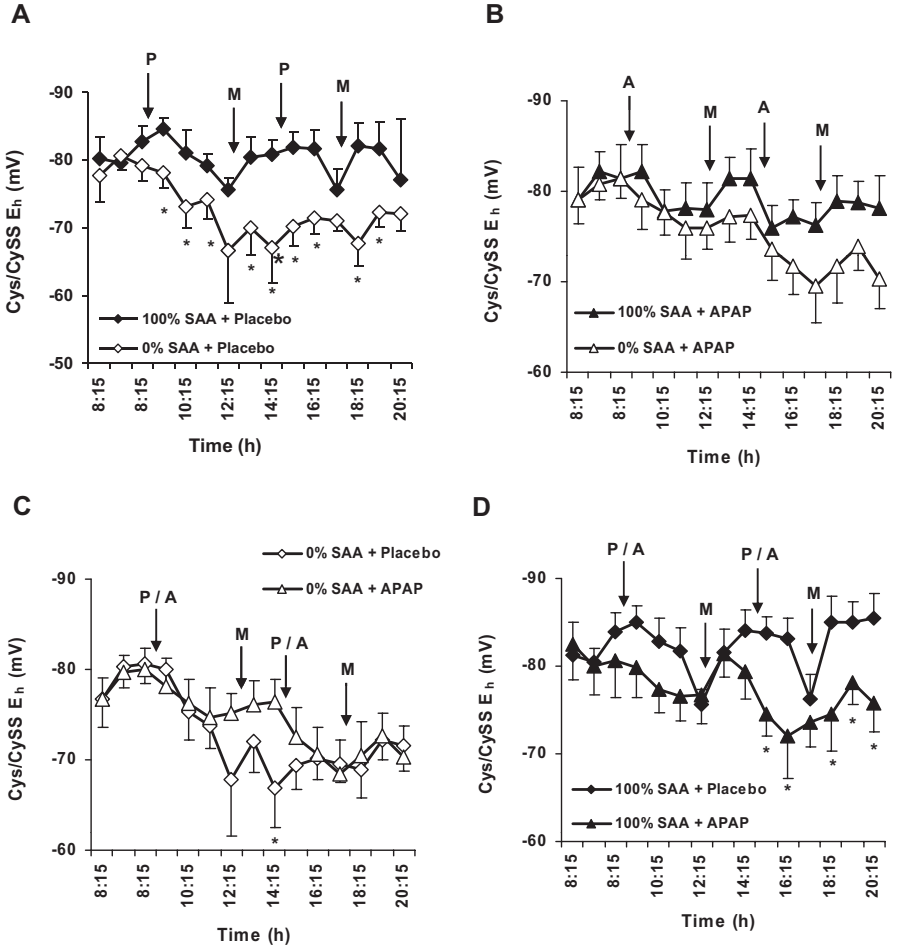

Fig. 4. Effect of SAA insufficiency and APAP administration on plasma Cys/CySS redox potential (E_h CySS) in healthy persons aged 18 to 40 years. Plasma E_h CySS was calculated by using the Cys and CySS concentrations measured by HPLC and the Nernst equation. See legend to Fig. 1 for details. ∗, significance at $p < 0.05$.

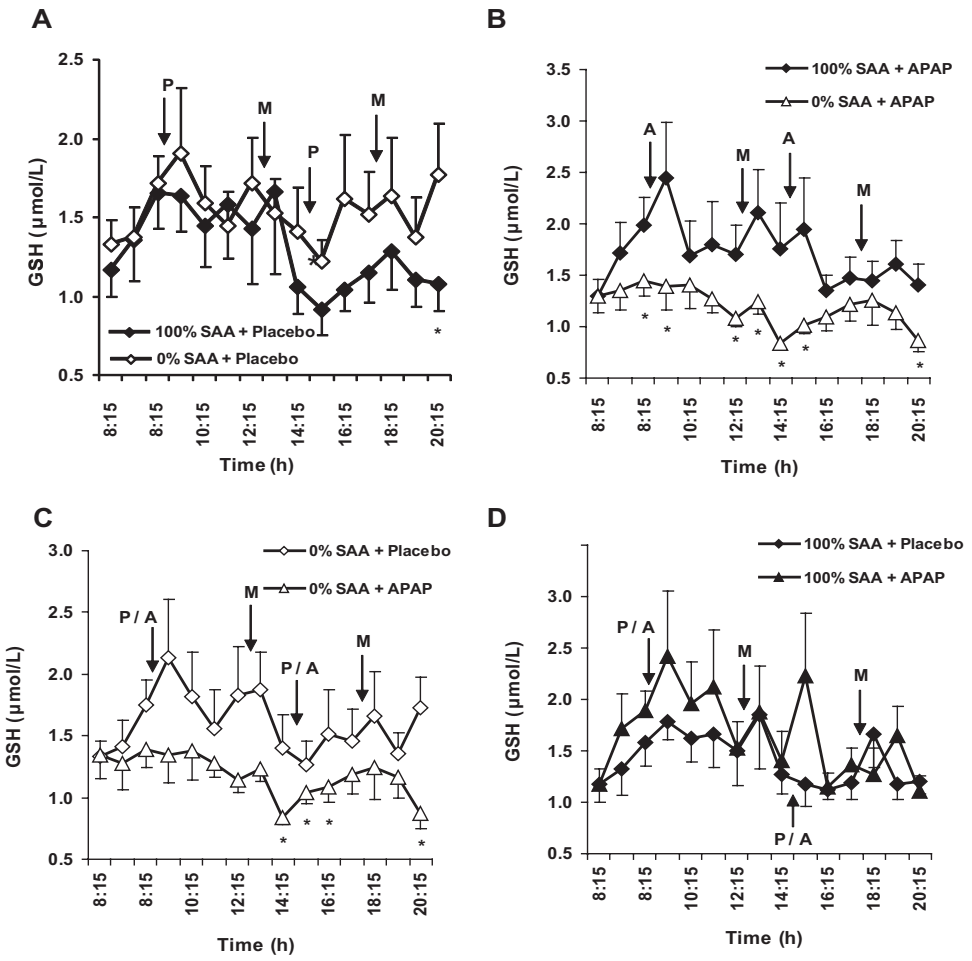

Fig. 5. Effect of SAA insufficiency and APAP administration on plasma GSH concentration in healthy persons aged 18 to 40 years. See legend to Fig. 1 for details. *, significance at $p < 0.05$.

Discussion

Previous research in rodents showed that consumption of a diet insufficient in SAA and/or prolonged ingestion of APAP causes a decrease in tissue Cys and GSH concentrations (Reicks et al., 1988; Price and Jollow, 1989). Plasma GSH is derived largely from hepatic release in a process thought to maintain supply of Cys throughout the body (Moriarty-Craige and Jones, 2004). However, the effects of altered SAA intake and therapeutic doses of APAP on extracellular redox potential have not previously been studied in humans. The present data show that mild SAA insufficiency significantly decreased plasma Cys concentration and caused more oxidized plasma E_h CySS value in humans. Under SAA insufficient conditions, therapeutic doses of APAP significantly increased plasma CySS concentration and decreased plasma GSH concentration. However, despite the changes caused by SAA insufficiency, therapeutic doses of APAP did not further oxidize plasma E_h CySS or E_h GSSG.

The observed decrease in plasma GSH caused by APAP suggests that APAP decreases hepatic GSH available for release into plasma. In a study of SAA insufficiency in human colon carcinoma (HT29) cells, Miller et al. (2002) showed that severe Cys deficiency decreased intracellular GSH concentration, oxidized E_h GSSG values, and inhibited cell proliferation. Addition of Cys resulted in a nearly complete recovery of intracellular GSH concentration and produced a rapid recovery from the oxidized conditions associated with Cys defi-

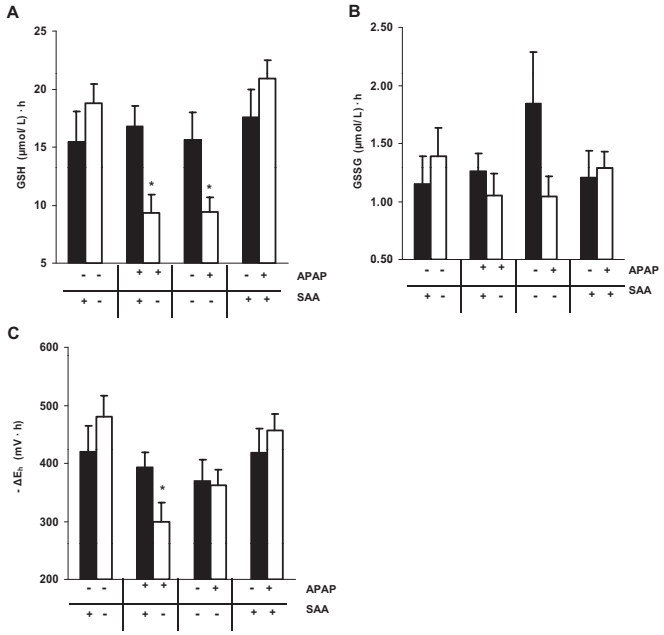

Fig. 6. AUC for plasma GSH and GSSG concentrations and GSH/GSSG redox potential (E_h GSSG) in a 2 × 2 design to study effects of SAA insufficiency and therapeutic doses of APAP. AUC values were calculated by using the trapezoidal rule for the individual time courses used to create the summary data for: A, GSH (Fig. 5); B, GSSG (Fig. 7), and C, E_hGSSG (Fig. 8). See Fig. 2 for additional details.

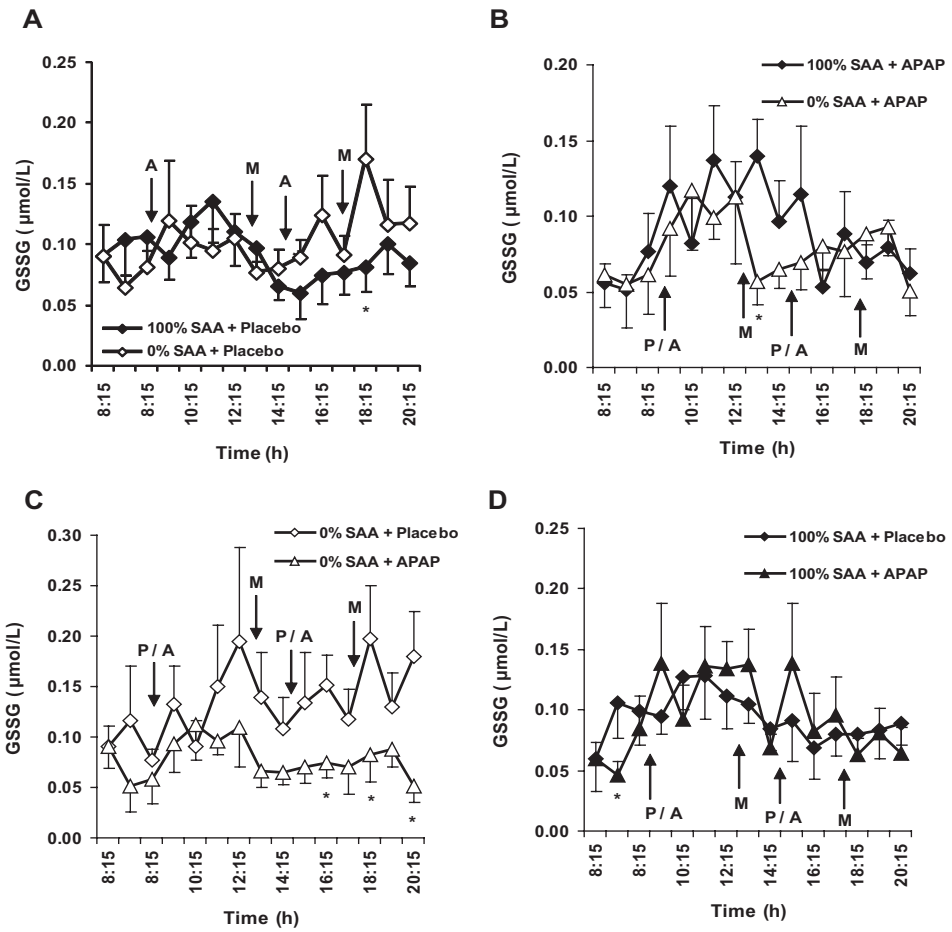

Fig. 7. Effect of SAA insufficiency and APAP administration on plasma GSSG concentration in healthy persons aged 18 to 40 years. See legend to Fig. 1 for details. *, significance at $p < 0.05$.

ciency (Miller et al., 2002). This earlier study provided a link between limited SAA intake and oxidation of intracellular E_h GSSG in vitro. Nkabyo et al., (2006) provided evidence that redox changes occur in vivo in response to SAA insufficiency in both the cellular and the extracellular redox compartments. Results showed that in adult rats 7 days of dietary SAA insufficiency caused a decrease in tissue and plasma GSH concentrations, an increase in plasma GSSG, and oxidation of the plasma and intracellular E_h GSSG values. In addition, dietary SAA insufficiency caused a marked decrease in plasma Cys and CySS concentrations, leading to an oxidation of plasma E_h CySS values (Nkabyo et al., 2006). Dietary supplementation of SAA resulted in an increase in plasma Cys concentration and more reduced plasma E_h CySS values (Nkabyo et al., 2006). Those studies in rats, coupled with the present data in humans, show that the level of dietary SAA intake can alter the redox status of plasma Cys/CySS and intracellular GSH/GSSG. The present study adds to this knowledge by showing that acute therapeutic doses of APAP do not increase the extent of change caused by SAA insufficiency in humans (Figs. 4C and 8C).

APAP metabolism critically depends on SAA availability, and with toxic doses, the extent of toxicity is exacerbated by SAA limitation (Price and Jollow, 1989). APAP toxicity is initiated by the metabolism of APAP to *N*-acetyl-p-benzoquinone imine (Dong et al., 2000; James et al., 2003). *N*-acetyl-p-benzoquinone imine depletes hepatic GSH and subsequently binds to cellular proteins to induce toxicity and oxidative stress (Mitchell et al., 1973; Jaeschke et al., 2003). Price and Jollow (1989) investigated whether limitation of SAA consumption would potentiate APAP hepatotoxicity in rats. The results showed that consumption of an SAA-deficient diet and acute APAP administration decreased hepatic GSH concentrations and increased the incidence and severity of APAP-induced toxicity (Price and Jollow, 1989). The present data show that a therapeutic, nontoxic dose of APAP significantly decreases plasma GSH concentrations in humans consuming a SAA-free diet (Fig. 5B). These results are consistent with the rodent studies and further show that effects on the GSH system are apparent in humans even with normal therapeutic doses.

Effects on plasma Cys and GSH caused by normal therapeutic doses of APAP are somewhat unexpected given the large pool of GSH in the liver. Reference values for hepatic GSH content are 3 to 10 mM or 1.5 to 4.9 g GSH for a 1.6-kg liver. The respective content of Cys within GSH is 0.58 to 1.9 g. Assuming that all dietary SAA intake is available for conversion to Cys, the deficit caused by 2 days without dietary SAA is twice the RDA, or 3.8 g Cys equivalents. Consequently, the potential deficit caused by 2 days without SAA (3.8 g) is large relative to the Cys content in hepatic GSH (0.58 to 1.9 g). However, this comparison does not include the total body GSH, which is available because of interorgan GSH/cysteine cycling. Assuming 1 mM GSH in 50 liters of body water, the body contains approximately 15 g of GSH, which contains 5.8 g of Cys. In the present study, approximately 0.6 g of SAA would be needed to supply GSH and sulfate for conjugation of 2 g of APAP. Consequently, the

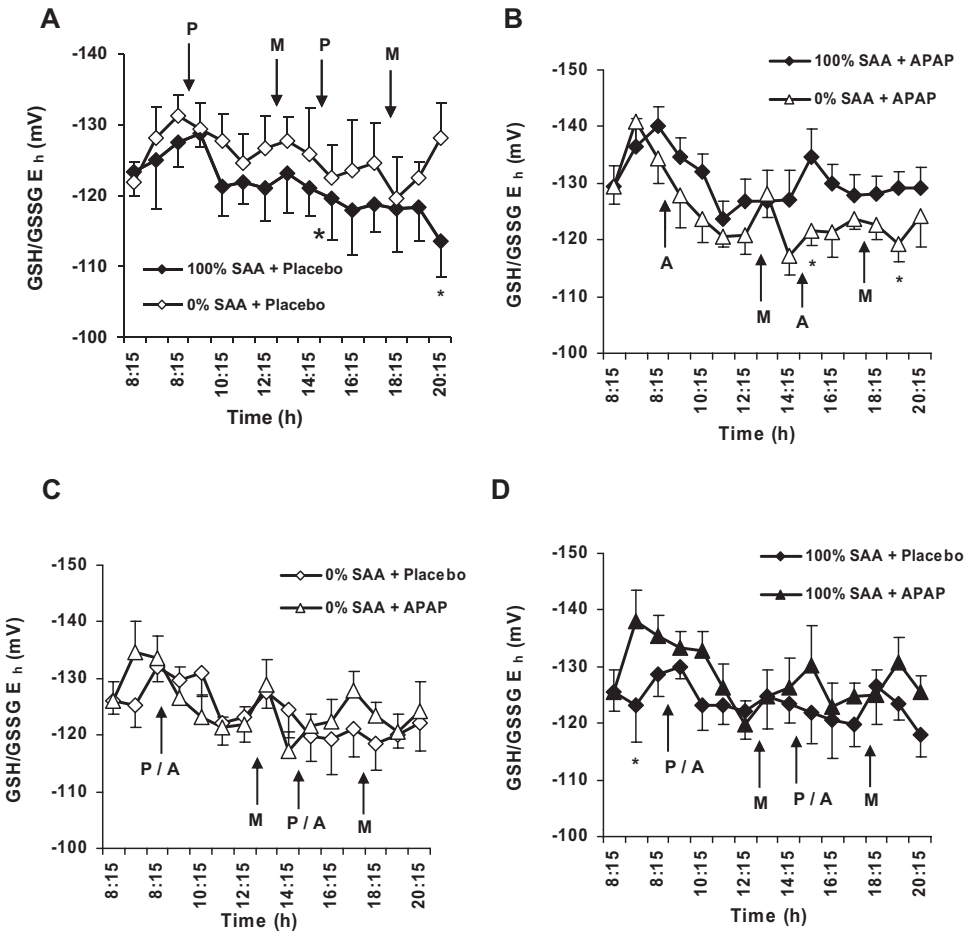

Fig. 8. Effect of SAA insufficiency and APAP administration on plasma GSH/GSSG redox potential (E_hGSSG). Plasma E_h GSSG was calculated by using the GSH and GSSG concentrations measured by HPLC and the Nernst equation. See legend to Fig. 1 for additional details. *, significance at $p < 0.05$.

doses administered consume the equivalent of approximately 10% of the Cys contained in the total body pool of GSH.

Recent studies show that more oxidized values for plasma E_h CySS and E_h GSSG are associated with aging and risk factors for cardiovascular disease (Go and Jones, 2008). In particular, an oxidation of 10 mV in plasma E_hCySS is associated with persistent atrial fibrillation (Neuman et al., 2007) and increased proinflammatory signaling (Iyer et al., 2009). Protein domains of receptors, transporters, and adhesion molecules on the surface of cells are exposed to the extracellular Cys/CySS pool, and 10-mV oxidation is sufficient to cause a 2-fold change in the reduced/oxidized ratio of dithiol-disulfide couples. The present results show that APAP can cause 10-mV oxidation of E_h CySS (Fig. 4D), suggesting that APAP could affect redox signaling mediated via E_hCySS. No relevant in vivo data are available, but in vitro data show that an oxidized extracellular E_hCySS in cell culture activates proinflammatory signaling (Go and Jones, 2005; Go et al., 2010), profibrotic signaling (Ramirez et al., 2007), stress signaling (Go et al., 2009), and apoptotic signaling (Jiang et al., 2005). Although speculative, the present data suggest a number of unanticipated effects of therapeutic use of APAP could occur as a consequence of effects on plasma E_hCySS.

Under SAA-insufficient conditions, APAP does not oxidize either Cys/CySS or GSH/GSSG (Fig. 4C). This observation is potentially relevant to situations such as the anorexia associated with illness in which patients may consume diets with inadequate or no protein (and thus insufficient SAA) for several days. The results suggest that use of APAP under these conditions may not cause further oxidative changes, as measured in plasma. A limitation of the current study was the short duration of SAA depletion and the limitation to only two doses of APAP in otherwise healthy adult subjects. Thus, studies of the effects of longer periods of APAP use, particularly in patients with illness consuming inadequate oral diets would be of interest, as would studies in children, in which APAP is commonly used during viral and other febrile illnesses. In contrast to the effects on the Cys couple, we found that APAP significantly decreased plasma GSH during the SAA-insufficient period in our subjects (Fig. 5C). Because the liver is a major source of plasma GSH, studies that evaluate the GSH response to APAP in patients with impaired hepatic function or with longer periods of SAA insufficiency would also be of interest.

In conclusion, the present research shows that therapeutic doses of APAP result in a more oxidized plasma Cys/CySS pool. These changes occur in humans irrespective of short-term changes in SAA intake. SAA insufficiency or APAP administration decreased plasma GSH concentration but had no effect on E_h GSSG.

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